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Pollen starch reserves in tomato relatives: Ecophysiological implications

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Abstract
The presence or absence of starch in microspore development and in pollen grains was recorded in eleven wild tomato species (*Solanum* sect. *Lycopersicon*) and two close relatives (*S. lycopersicoides* and *S. sitiens*). In all the species starch started to accumulate in the early microspore bicellular stage and continued until the cytoplasm was filled. At flower anthesis, pollen grains were mostly starchless in the wild tomatoes, except in *S. pennellii*, which had starchy pollen. Starchy pollen is also present in the two related species. The latter two species had larger pollen grains and grow in drier environments than the other species. The heterogeneity of pollen starch content among all these species, supposed to have the same pollination mechanism, is a new finding supporting the idea that starch content and pollination mechanism do not necessarily influence each other. The presence of starchy pollen in the self-incompatible species, which grow in the driest environments, raises questions regarding the relationship between carbohydrates content and pollen survival.

Keywords: *Starchy pollen, starchless pollen, pollen survival, wild tomatoes*

Pollen grains have two main reserves, starch in amyloplasts and lipids in sphaerosomes (Pacini & Hesse, 2004). The presence of one or the other has been related to the pollen size and pollination mechanism (Baker & Baker, 1979). However, as the number of species studied has increased, several exceptions have emerged (e.g. Zona, 2001). This includes some evidence that suggests that pollen starch content does not grealy influence the pollination mode and vice versa (Roulston & Buchmann, 2000).

Species of *Solanum* L., among them tomatoes (*Solanum* sect. *Lycopersicon*), have buzz-pollinated flowers that follow the so-called *Solanum*-type (Buchmann, 1983; Endress, 1994). In addition to the morphological singularities of the androecium (e.g. connivent anthers, opening through apical pores; Buchmann, 1983), the *Solanum*-type flowers also have particular features regarding pollen shape, size, colour, and reserve substances (Buchmann, 1983). Pollen grains are supposed to have no starch in *Solanum*-type flowers (Buchmann, 1983). A study in several *Solanum* species, including the cultivated tomato *Solanum lycopersicum* L., revealed that their pollen grains are indeed starchless (Buchmann, 1986). However, during a histogenetic study of the anther, differences in pollen starch content appeared among wild tomatoes (Carizzo García: pers obs).

The aim of the present work is to document the variability of starch content throughout microspore and pollen development in the wild tomatoes (*Solanum* sect. *Lycopersicon*) and two close relatives (*S. lycopersicoides* Dunal and *S. sitiens* I. M. Johnst.), and to analyse its possible implications.

Material and methods

Eleven wild tomato species and two species closely related to the wild tomatoes were studied (see Specimens Investigated list, and Spooner et al., 2005, for an overview of phylogenetic relationships). Most materials were obtained from plants grown in

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greenhouses, and were collected at different times during summer.

Buds and flowers from all species were fixed in a mixture of formalin (10%), acetic acid (5%), and ethanol 70% (85%). For all species, starch content was studied in a series of developmental stages from microspores just released from the tetrads to mature pollen. Three microspore stages were defined, namely unicellular microspore, early bicellular microspore (where the generative cell is still attached to the intine) and late bicellular microspore (where the generative cell is free within the vegetative cell). Pollen belonging to flowers of various positions along the inflorescence was analysed in order to cover different stages of anthesis. For *Solanum pennellii* var. *puberulum* and *S. lycopersicoides* flowers were picked for each day of the three days of anthesis, and their pollen was analysed separately (i.e. pollen of different ages). The two varieties of *S. pennellii* have anthers of three different lengths, therefore pollen samples from all three kind of anthers were observed independently.

Anthers were detached from flowers and buds, washed with water, and then cut open to separate their content on a slide. A drop of iodine-potassium iodine (IKI) was added to the microspores and pollen to reveal the presence of starch (Johansen, 1940), and the material was immediately observed using light microscope (bright field, BF). Starch stained dark bluish-brown. Starch presence or absence was recorded. Even though the amount of starch was not quantified, differences were noted between species, according to the coloration intensity. The microspore cellular stage was defined observing the samples under differential interference contrast microscope (DIC).

Anthers of all species were picked from flower buds of different sizes and embedded in Paraplast, cut with a microtome in sections 10–12 μm thick, and stained with haematoxylin, safranin and fast green. The microspore cellular stage was also determined in these samples, while the presence of starch was revealed using polarised light.

Pollen volume was calculated in fixed hydrated material for all the species to reveal possible differences. In addition, the volume of microspores was calculated at different stages for *S. corneliomulleri*, *S. neorickii*, *S. pennellii* var. *puberulum* and *S. lycopersicoides*, to show the size change during development. As the material was preserved in a liquid fixative, i.e. microspores and pollen grains were spherical, the volume was calculated with the formula $\frac{4}{3}\pi r^3$, where r is the radius. A total of 100 microspores and pollen grains were measured for each stage and species.

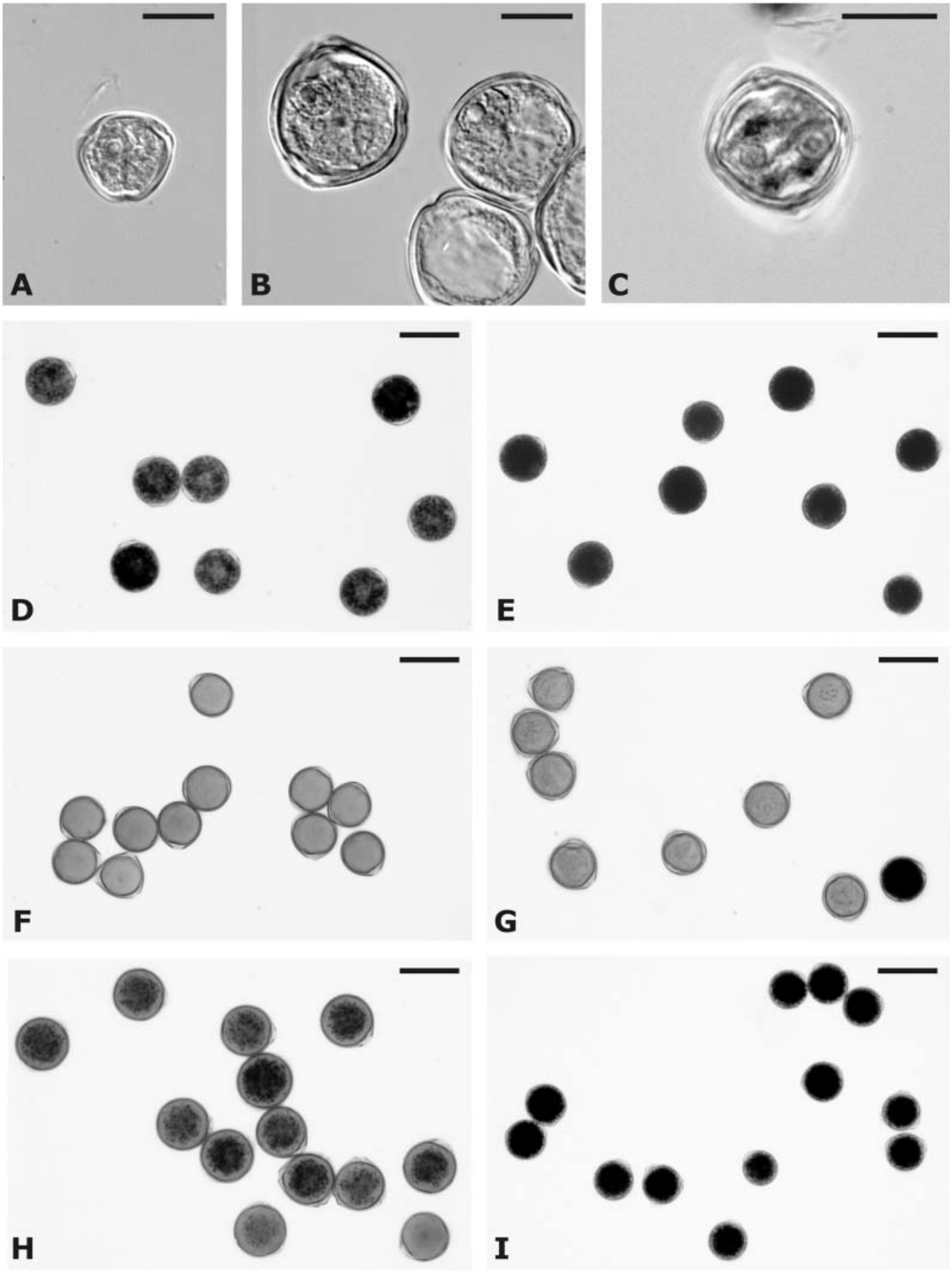
Results

None of the species showed starch detectable with the methods employed at the early unicellular microspore stage (Figures 1A, B & 2A). Starch started to accumulate in the cytoplasm during the early bicellular microspore stage (Figure 2B). The amount of starch increased during the late bicellular stage (Figure 1C) until it filled the cytoplasm, close to the flower opening, where the microspores appeared densely stained (Figures 1D, E & 2C). At flower anthesis, when anthers were open and pollen was mature, the latter was either starchless or starchy (Figures 1F–I & 2D, E), depending on the species.

Starch content diminished quickly towards anthesis in *S. arcanum, S. cheesmaniae, S. chilense, S. chmielewskii, S. corneliomulleri, S. galapagense, S. habrochaites, S. neorickii, S. pimpinellifolium, and S. peruvianum*. A high amount of starch was detected in the microspores up to the largest bud before anthesis (Figure 1D), but no starch was present in pollen grains (Figure 1F), except in a few of them (Figure 1G). The presence of pollen grains with a variable amount of starch was more frequent in S. *chilense* (between 10–15%; Figure 1G) than in the other nine species. For the ten species, the characteristics mentioned were uniform in all the flowers along the inflorescence.

In the two varieties of *S. pennellii*, starch content in the microspores declined slightly towards anthesis. This species presented mainly starchy pollen, though the amount of starch was variable among pollen grains (Figure 1H). Indeed, some pollen grains were densely stained revealing a high amount of starch, in other pollen grains the coloration was lighter, allowing the recognition of individual starch grains (Figure 1H), while a small number of pollen grains were starchless. The characteristics mentioned were uniform among the anthers of different sizes in both varieties of *S. pennellii*, and also among flowers from different positions in the inflorescence and from each day of anthesis in *S. pennellii* var. *puberulum*.

Figure 1. Starch content in microspores and pollen grains in wild tomatoes and relatives. **A**, **B**. Unicellular microspores devoid of starch (DIC). **A**. *Solanum galapagense*; **B**. *S. sitiens*. **C**. Microspores of *S. corneliomulleri* at bicellular stage, when starch (black spots) has started to accumulate (DIC). **D**, **E**. Late bicellular microspores filled with starch (BF). **D**. *S. peruvianum*; **E**. *S. lycopersicoides*. **F**. Starchless pollen of *S. pimpinellifolium* (BF). **G**. Pollen mostly starchless of *S. chilense*; note the presence of a pollen grains filled with starch (in black colour) (BF). **H**. Starchy pollen grains of *S. pennellii* var. *puberulum*; note the variable amount of starch (in black colour) among pollen grains (BF). **I**. Starchy pollen of *S. lycopersicoides* (BF). All figures stained with iodine-potassium iodine. Scale bars – 15 μm (A, B); 10 μm (C); 25 μm (D, F, G, H); 50 μm (E, I).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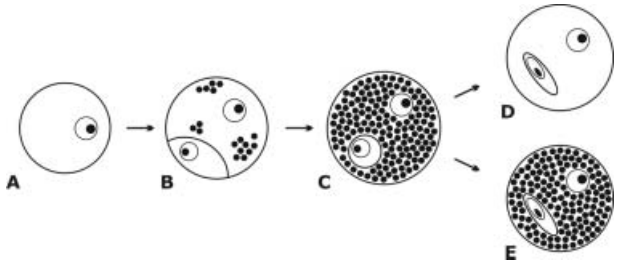

Figure 2. Semi-diagrammatic scheme representing stages of microspore development and pollen grains, showing the fluctuations and differences in starch content in wild tomatoes and close relatives. **A**. Starchless unicellular microspore. **B**. Beginning of starch deposition in early bicellular microspore. **C**. Late bicellular microspore completely filled with starch. **D**. Starchless pollen grain common to most wild tomatoes. **E**. Starchy pollen grain present in *Solanum pennellii*, *S. lycopersicoides* and *S. sitiens*.

All pollen grains of *S. lycopersicoides* and *S. sitiens* had abundant starch evidenced by the strong colour obtained for all flowers (Figure 1I). Regarding *S. lycopersicoides*, pollen was starchy in all samples observed from flowers of different days of anthesis.

Pollen volume ranged from ca. 2 000 to ca. 5 500 μm^3 among wild tomatoes (diameter ranging from 17.5 μm in *S. neorickii* to 21.9 μm in *S. pennellii*), those of both varieties of *S. pennellii* being the largest (Figure 3). Pollen grain volume in *S. lycopersicoides* and *S. sitiens* was around five times bigger than the average pollen volume of the wild tomatoes (Figure 3). Microspore volume increased during development with a peak at the late bicellular stage (Figure 4), when they were completely filled with starch. The volume decreased from the late microspores to the pollen grains, regardless whether they were starchless or starchy (Figure 4). Although the pattern of volume change was the same in the four species analysed, the microspore size was larger in *S. lycopersicoides* than in the wild tomatoes in every stage considered, in concordance with the larger size of the pollen grains.

Discussion

Starch accumulated in the microspores during development, beginning at the early bicellular microspore stage in all 13 species studied here, as earlier reported for *S. lycopersicum* (Polowick & Sawhney, 1993; Pressman et al., 2002) and *S. peruvianum* (although two peaks of amylogenesis have been reported in this species; Pacini & Juniper, 1984; Pacini & Viegi, 1995). Despite the initial coincidence in starch metabolism, it varied towards flower anthesis because starch can either disappear or be retained. Thus, two groups of species can be distinguished according to the pollen starch content:

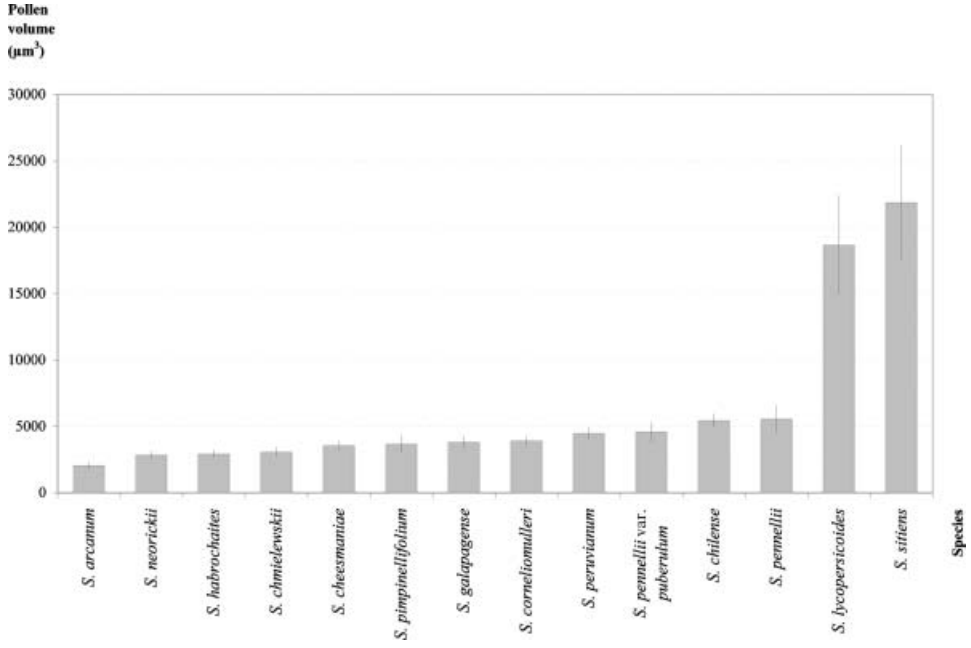

Figure 3. Pollen grains volume in wild tomatoes and close relatives. Starchy pollen grains in *Solanum lycopersicoides*, *S. sitiens*, *S. pennellii* and *S. pennellii* var. *puberulum*, starchless pollen grains in the remaining species.

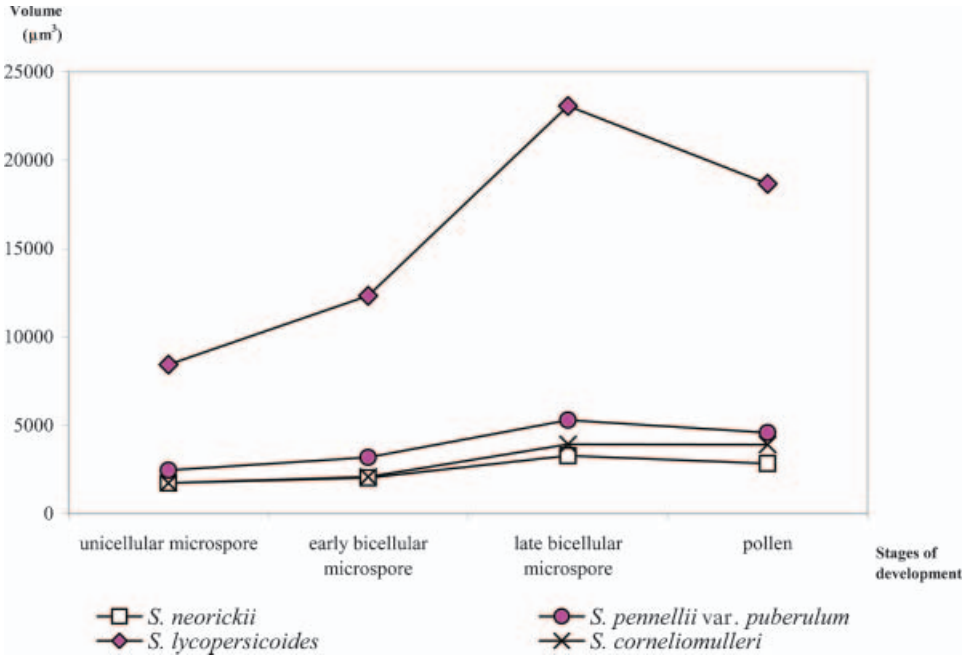

Figure 4. Volume of microspores at different stages and pollen grains in several wild tomatoes and a close relative species. Starchless pollen grains in *Solanum corneliomulleri* (×) and *S. neorickii* (□), starchy pollen grains in *S. pennellii* var. *puberulum* (●) and *S. lycopersicoides* (◆).

species with starchless pollen and species with starchy pollen. The first group comprises tomatoes '*sensu stricto*' (*S. arcanum*, *S. cheesmaniae*, *S. chilense*, *S. chmielewskii*, *S. corneliomulleri*, *S. galapagense*, *S. habrochaites*, *S. neorickii*, *S. pimpinellifolium* and *S. peruvianum*), including the cultivated tomato (*S. lycopersicum*), i.e. the former *Lycopersicon* taxa (after Rick, 1979, and Taylor, 1986). The second group includes the two species related to the tomato group, *S. lycopersicoides* and *S. sitiens*, and the two varieties of the controversial *S. pennellii* (cf. Rick, 1979, and Taylor, 1986). The androecium of *S. pennellii* differs in several features from the other tomatoes (Carrizo García, 2003), and as shown here the pollen starch is another feature that may be used to distinguish the species.

Regarding pollen size, it has been observed in species of other families that starchy pollen grains were larger (Baker & Baker, 1979; Grayum, 1985; Zona, 2001; López et al., 2006). It may be noted that the two related species of the tomato group, which had exclusively starchy pollen, also had larger pollen grains. However, starch content and pollen size may not be directly correlated since the starchy grains of *S. pennellii* are clearly smaller than those of *S. lycopersicoides* and *S. sitiens* and more similar in size to pollen of other tomatoes (although the largest). López et al. (2006) suggested that pollen below a critical size might not be able to store starch to sustain pollen tube growth, but other causes could perhaps explain the trait observed in *Solanum* (see below).

Starch presence in pollen has also been related to pollination mechanism (Baker & Baker, 1979), but this was recently questioned by Roulston and Buchmann (2000). The variation observed among the *Solanum* species studied here further indicates that such a relationship may not be an universal rule. Although there is a tendency to the presence of starchless pollen in *Solanum* as documented previously (Buchmann, 1986; Franchi et al., 1996; Passarelli, 1999; Roulston & Buchmann, 2000; Pressman et al. 2002), starchy pollen grains have also been found in the genus.

Roulston and Buchmann (2000) suggested that the relation between sugars and desiccation could better account for the variation in starch content. This idea is in accordance with the hypothesis of Franchi et al. (1996). These authors have suggested that plants from dry environments have starchless pollen, which would be more resistent to dehydration than starchy pollen. The evidence that sucrose would help to preserve membrane stability (Hoekstra et al., 1992, 2001) and to keep pollen turgor pressure (Pacini & Hesse, 2004), both important for pollen survival (Pacini & Hesse, 2004), and that the sucrose level raises while the amount of starch diminishes during microspore development (Hoekstra & van Roekel, 1988; Speranza et al., 1997), support Franchi et al. (1996) idea. In this regard, the *Solanums* spp studied here set another exception. Since wild tomatoes are from xerophytic environments along the west cost of South America (Peru, Ecuador, and northern Chile)

and the Galapagos Islands (Luckwill, 1943; Spooner et al., 2005), they would be expected to have starchless pollen. Actually, most tomatoes had starchless pollen, except for *S. pennellii*. *S. lycopersicoides* and *S. sitiens* which occur in drier environments in southern Peru and northern Chile (Rick, 1988). Interestingly both species, as well as *S. pennellii*, are self-incompatible (Rick, 1979; Rick, 1988), which means that pollen must survive long enough to be transferred by a pollinator to a compatible flower. Nevertheless, the three species have starchy pollen. Even though most tomatoes matched the hypothesis of Franchi et al. (1996), *S. pennellii*, and the related *S. lycopersicoides* and *S. sitiens* do not. Then, if sucrose works as it has been suggested, maybe the presence of starch does not imply a low level of sucrose in these cases, so the pollen could resist and survive even in a dry environment, or the pollen has another mechanism that facilitates its survival. In fact, an interesting difference between two *Helleborus* species that cohabit and set flowers during the same period has been demonstrated. Pollen had similar percentages of viability and similar levels of sucrose in both species, but in one of the species pollen grains were starchless while they were starchy in the other (Vesprini et al., 2002). Thus, the presence of starch in pollen grains may not necessarily imply short lived pollen. A study of pollen viability, longevity and carbohydrates in live specimens would help to understand the significance of the variations observed among the species studied.

Conclusion

The study of pollen starch content presented here provides new evidence on the complex relationship between pollen metabolism and the constrains imposed by the reproductive features of a species (including pollination mechanism) and the environment in which the species is growing, and demonstrates that considerable variation is present even among closely related taxa.

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Species investigated

BGN – Botanical and Experimental Garden of Nijmegen, The Netherlands; TGRC – C.M. Rick Tomato Genetics Resource Center, USA

Wild tomatoes

Solanum arcanum Peralta. C. Carrizo García; BGN, N° 974750061

Solanum cheesmaniae (Riley) Fosberg. R. Chetelat; TGRC, LA 0166

Solanum chilense (Dunal) Reiche. R. Chetelat; TGRC, LA 1970

Solanum chmielewskii (C.M. Rick, E. Kesicki, J.E. Fobes et M. Holle) D.M. Spooner, G.J. Anderson et R.K. Jansen. R. Chetelat; TGRC, LA 1330

Solanum corneliomulleri J.F. Macbr. C. Carrizo García; BGN, N° 974750064

Solanum galapagense S. Darwin et Peralta. R. Chetelat; TGRC, LA 0317

Solanum habrochaites S. Knapp et D.M. Spooner. C. Carrizo García; BGN, N° 944750111. R. Chetelat; TGRC, LA 2099

Solanum neorickii D.M. Spooner, G.J. Anderson et R.K. Jansen. A.T. Hunziker; BGN, N° 974750067

Solanum pennellii Correll. A.T. Hunziker; BGN, N° 964750063. C. Carrizo García; BGN, CORD, f. 4024

Solanum pennellii var. *puberulum* Correll. C. Carrizo García; BGN, LA 1926. C. Carrizo García; BGN, LA 750

Solanum peruvianum L. C. Carrizo García; BGN, N° 914750145

Solanum pimpinellifolium L. A.T. Hunziker 25483; Peru, Dpto. La Libertad. C. Carrizo García; BGN, N° 934750008.

Relatives of the tomato group

Solanum lycopersicoides Dunal. C. Carrizo García; BGN, N° 974750042

Solanum sitiens I. M. Johnst. R. Chetelat; TGRC, LA 1974

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